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In vitro Drug Release Prediction of Hydrochlorothiazide Modified Release Tablet using Wagner Nelson Method and Deconvolution Approach:

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Abstract : Background: Immediate release formulation of Hydrochlorothiazide is available in market. Cmax achieved at 2 h, duration of action persist for 6-12 h, resulting in multiple dosing to maintain plasma concentration and *in vivo* activity. **Objective:** The objective of research work was to design modified release tablets of Hydrochlorothiazide 25 mg with the intention of once a day dosing. Cmax will achieve after 10 to 12 h of administration. This controlled release will constantly induce diuretic activity for whole night and day especially during early morning hours, resulting in decreased blood volume, reduced cardiac output and controlled blood pressure. When patient awakes up in morning, blood pressure will be comparatively low thus reducing the frequency of cardiac arrest.**Method:** Here an attempt has been made to kinetically calculate required in-vitro dissolution profile by deconvolution method using Wagner–nelson equation. Plasma concentration time profile of immediate release tablet 12.5 mg is available in literature and that of modified release formulation 25 mg is calculated such that Area under curve of modified release tablet matches with that of two immediate release tablets of 12.5 mg along with Cmax and Kel, thus meeting the criteria for bio-equivalency. Tmax will be delayed from 2 h to approximately 10 h. **Results:** In-vivo dissolution profile is calculated from the equation of "fraction of drug absorbed". Perfect IVIVC is matched when value of slope is 1, intercept is 0 and correlation coefficient (R^2) 0.99. **Conclusion:** This in-vitro profile will be used further to develop modified release formulation.

Keywords : Area under curve, Cmax, Elimination rate constant, Deconvolution, Wagner – Nelson equation, IVIVC.

Introduction

Heart Attack

It is known for decades that the most dangerous time for all kind of cardiovascular emergencies including and not limited to heart attack, sudden cardiac death, failure in heart's electrical system rupture of the aorta, pulmonary embolism and stroke, are the early morning hours and during the last phase of sleep[1 – 4].

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Results from various studies suggest that most of the sudden deaths occur from 7 to 11 AM with lowest occurrence during sleep[5, 6]. Cardiovascular system has oscillatory nature which follows a routine pattern it exhibit circadian changes. Imbalance between decreased myocardial oxygen supply (less oxygen available) and increased myocardial oxygen demand (more oxygen is needed in our heart) or sometimes both results in Heart attack. Physical activities of first hours of day starting from waking up require increased myocardial oxygen support. Adrenal hormone cortisol concentration increases in blood resulting increased blood – pressure along with elevated blood sugar levels. Further elevation of blood pressure and heart rate occurs due to catecholamines (adrenaline and noradrenaline), which are at their peak concentration when a person wake up in morning[7].

Hydrochlorothiazide

Hydrochlorothiazide is most commonly prescribed anti-hypertensive and for more than three decades. The prescription pattern of HCTZ has been heavily influenced by the eight reports of Joint national committee for prevention, detection, evaluation and treatment of High blood pressure. All reports recommends "thiazide" or "thiazide-like drugs" or 'thiazide type diuretics" as first line therapy with usual dose ranging from 12.5 to 50 mg per day in single or divided dose as recommended[8]. 2017 guideline of Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults recommends HCTZas one of the primary agents to be used as oral antihypertensive drugs[9]. The more recent JNC reports have recommended that low dose thiazide and thiazide-like diuretics should be used as initial therapy in hypertensive patients.

HCTZ is available as immediate release tablet with multiple strengths (12.5mg to 100 mg). There are several combination also available of HCTZ immediate release tablet with antihypertensive drugs. Hydrochlorothiazide is rapidly absorbed from gastrointestinal tract. After oral administration onset of action occurs in 2 h, peak effect is observed at approximately 4 h. Duration of action persists for approximately 6 to 12 h after each dose[10, 11].HCTZ belongs to BCS Class II molecule with poor water solubility and good permeability. For BCS Class I & II molecules, permeability is high, hence fraction of drug dissolved (FRD) is equal to fraction of drug absorbed (FRA).

IVIVC

In-vitro In-vivo correlation is a mathematical relationship between *in vitro* properties of a dosage form with its *in vivo* performance. The i*n-vitro* release data API serve as characteristic *in vitro* property, while the *In vivo* performance is generally represented by the time course of the plasma concentration of the active substance. Scientific treatment of these i*nvitro and invivo* data results in correlations. Dissolution rate is usually measured for solid oral dosage forms which characterize *in vitro* release. Linear and nonlinear mathematical correlation can be expressed to characterize *in vitro* and *in vivo* relationship. Direct correlation of plasma concentration with *in vitro* release cannot be done. Pharmacokinetic compartment model analysis or linear system analysis is used to convert plasma concentration to *in vivo* release or absorption data. [12].

There are three levels of IVIVC which are defined: Level A which is considered most informative represents point to point relationship between in-vitro dissolution data and in-vivo plasma drug concentration time profile. It is also recommended by FDA. Level B IVIVC is not a point to point correlation and utilizes the principles of statistical moment theory where MDT (mean in-vitro dissolution time) is compared with MDT (mean in-vivo dissolution time) or MRT (mean in-vivo resident time). Level C is a single point correlation and compares one dissolution time point with one mean pharmacokinetic parameter such as C_{max}, T_{max}, AUC. This correlation is considered as the weakest among all as only a partial relationship is established.

Convolution is a combination of two mathematical functions to create third function.it is used to find out plasma drug concentration time profile from a unit input response and a given drug input rate. It can be performed by various available techniques including Laplace Transform technique, Analytical method or convolution by integral. Whereas Deconvolution is an exactly opposite mathematical procedure to convolution. Deconvolution method is used to determine *in vivo* drug dissolution using unique input response and plasma concentration time profile. This concept is very helpful in pharmacokinetics to determine drug input rates from available plasma drug profile. One of the methods to develop level A correlation is to estimate the *in vivo* absorption or dissolution time course using an appropriate deconvolution technique such as Wagnor – Nelson

procedure, Loo-Riegelman method or numerical deconvolution. Wagner – Nelson is less complicated than Loo –Riegelman as there is no need of intravenous data, however both methods are model dependent [13]. According to Wagner-Nelson method, the cumulative fraction of drug absorbed at time t is calculated from equation as follows.

$$F_T = \frac{C_T + K_E \int_0^T Cdt}{K_E \int_0^\infty Cdt}$$

Where F_T is fraction of dose absorbed at time t, C_T concentration of drug at time t, K_E is elimination rate constant of drug, integration of concentration of drug from time '0' to 't' is area under curve from time '0' to 't' and integration of concentration of drug from time '0' to '∞' is area under curve from time '0' to 'infinity'.

Experimental:

Calculation of AUC & C_{max} for Hydrochlorothiazide IR Tablets:

The pharmacokinetic parameters of two oral formulations of 20/12.5 mg tablets of enalapril/ hydrochlorothiazide; Penopril as test and other commercially available preparation as reference were compared in an open-label randomized single oral dose two-period cross-over design to 24 healthy volunteers under fasting conditions. Pharmacokinetic parameters of 12.5 mg hydrochlorothiazide were used from this study[14]. Similar data was observed in Hydrochlorothiazide 12.5 mg tablet[15]. The results of two studies suggest that there is no major change in pharmacokinetics of hydrochlorothiazide 12.5 mg when given alone or in combination. The mean pharmacokinetic parameters are compiled in Table 1.

Table 1: Mean pharmacokinetic parameters of hydrochlorothiazide 12.5 mg.

	$AUC_{0-\infty}$ (ng x h/ml)	AUC_{0-t} (ng x h/ml)	C_{max} (ng/ml)	T_{max} (h)	$T_{1/2}$ (h)
N	24	24	24	24	24
Geom. Mean	566.0	516.2	81.4	1.8	5.0
Median	574.4	516.5	82.4	2.0	4.9
Mean	589.9	541.0	86.0	1.9	5.0
SD	170.7	167.3	27.7	0.6	1.0
Min	279.5	247.0	37.3	1.0	3.5
Max	968.7	912.6	137.0	2.7	7.4
CV(%)	28.9	30.9	32.2	31.7	20.5

Using this data Concentration of drug in ng/ml for HCTZ 12.5 mg single dose for 24 h was calculated. Values of concentration of drug at different time was found to be 0, 56, 77, 67, 58, 48, 37, 33, 23, 21, 17, 15, 12, 9, 8 and 7 ng/ml for 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 16, 20 and 24 h respectively. From single dose study, pharmacokinetic parameters of two doses of hydrochlorothiazide 12.5 mg dosed after every 12 his calculated. Concentration of drug up to 12 h will be same for 1st IR dose as mentioned above. After 12 h 8 ng/ml concentration of drug is left. Hence this remaining concentration will be added to second dose (drug accumulation). Elimination phase after 20 h is kept similar to first dose. Values of Concentration of two doses of IR tablet at different time was found to be 0, 56, 77, 67, 58, 48, 37, 33, 23, 21, 17, 15, 12, 68 (56+12), 89 (77+12), 79 (67+12), 70 (58+12), 60 (48+12), 49 (37+12), 45 (33+12), 35 (23+12), 21, 17, 15 and 12 ng/ml for 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 90, 20, 21, 22, 23 and 24 h respectively.

AUC is area under concentration time curve and is a measure of total systemic exposure of drug over time. It can be calculated from concentration – time data. AUC is not a primary pharmacokinetic parameter and is derived from clearance and dose. Area under curve is calculated using trapezoid method. This is also known as linear method and is most commonly used. Office of generic drugs at USFDA also prefers linear method. This method is used when concentration is increasing over time (absorption phase) and decreasing in poly-exponential fashion. In this method area of each trapezoid is calculated and then summed to get AUC of curve.Followingformula is used to calculate AUC where 'C' is the concentration of drug at two subsequent time

point 'T'. Refer Table 2 for AUC calculation of Hydrochlorothiazide immediate release tablet 12.5 mg single dose up to 24 h.

Table 2: Area under curve (AUC) calculation of hydrochlorothiazide immediate release tablet 12.5 mg single dose.

Time (h)	Concentration – 12.5 mg (ng/ml)	C1 + C2 (ng / ml)	Δ T (h)	AUC Linear (ng x h/ml)	AUC Total (ng x h/ml)
0	0	0	0	0.00	0.00
1	56	56	1	28.00	28.00
2	77	133	1	66.50	94.50
3	67	144	1	72.00	166.50
4	58	125	1	62.50	229.00
5	48	106	1	53.00	282.00
6	37	85	1	42.50	324.50
7	33	70	1	35.00	359.50
8	23	56	1	28.00	387.50
9	21	44	1	22.00	409.50
10	17	38	1	19.00	428.50
11	15	32	1	16.00	444.50
12	12	27	1	13.50	458.00
16	9	21	4	42.00	500.00
20	8	17	4	34.00	534.00
24	7	15	4	30.00	**564.00**
			Total	**564.00**	

Area trapezoid = ½ (C1 + C2) (T2 - T1)

= ½ (C1 + C2) Δ T

Total AUC was found to be 564.00. This is almost similar to AUC reported in literature[15]. AUC of two tablets of 12.5 mg immediate release tablet can be doubled i.e. 564 x 2 = 1128. For modified release formulation of 25 mg once a day dose, AUC should lie within 80% to 125% of two immediate release formulation of 12.5 mg which is 1128 i.e. 902 to 1410

Calculation of AUC and in-vivo dissolution of HCTZ Modified Release tablets:

Modified release formulation of HCTZ 25 mg is prepared considering two doses of 12.5 mg immediate release tablet. Hence in-vivo drug release profile for modified release tablet of 25 mg HCTZ is predicted to match

1. AUC of 2 immediate release tablets of 12.5 mg dosed at interval of 12 h.
2. Elimination phase similar to immediate release tablets.
3. C_{max} of modified release tablet is kept approximately 80-90% of immediate release tablet.
4. C_{max} of modified release tablet is achieved after approx. 10 h of dosing. When tablet will be taken at evening, C_{max} will be achieved at around 4 to 5 am.
5. T_{max} will be shifted from 2 h (IR tablet) to 10 h (MR tablet)

Along with AUC calculation, Fraction of dose absorbed is also calculated which is equal to in-vivo dissolution. Successive values of the right-hand side of Wagner – Nelson equation are calculated from the time of administration (t=0) to some-time after the peak in the blood level time plot (the area may be estimated by trapezoidal rule). The values progressively increased then reach a maximum or asymptotic value. When the individual values are expressed as percentage of the maximum values, the results are percent absorbed values to

various time T[16]. This is in-vivo drug dissolution. In-vivo dissolution calculation of modified release tablet 25 mg is shown in Table 3.

Table 3: Area under curve (AUC) and i*n vitro* dissolution calculation of hydrochlorothiazide modified release tablet 25 mg.

Time (h)	Concentration for 12.5 mg (ng/ml)	C1 + C2 (ng/ml)	Δ T (h)	AUC Linear (ng x h/ml)	AUC Total (ng x h/ml)	AUC X Kel	Conc + (AUC x Kel)	*In vivo* Dissolution (%)
0	0	0	0.00	0.00	0.00	0.00	0.00	0
1	18	18	1.00	9.00	9.00	0.68	18.68	16
2	30	48	1.00	24.00	33.00	2.51	32.51	28
3	39	69	1.00	34.50	67.50	5.13	44.13	37
4	45	84	1.00	42.00	109.50	8.32	53.32	45
5	51	96	1.00	48.00	157.50	11.97	62.97	53
6	56	107	1.00	53.50	211.00	16.04	72.04	61
7	60	116	1.00	58.00	269.00	20.44	80.44	68
8	62	122	1.00	61.00	330.00	25.08	87.08	74
9	64	126	1.00	63.00	393.00	29.87	93.87	80
10	66	130	1.00	65.00	458.00	34.81	100.81	85
11	66	132	1.00	66.00	524.00	39.82	105.82	90
12	66	132	1.00	66.00	590.00	44.84	110.84	94
13	65	131	1.00	65.50	655.50	49.82	114.82	97
14	64	129	1.00	64.50	720.00	54.72	118.72	101
15	63	127	1.00	63.50	783.50	59.55	122.55	104
16	60	123	1.00	61.50	845.00	64.22	124.22	105
17	55	115	1.00	57.50	902.50	68.59	123.59	105
18	49	104	1.00	52.00	954.50	72.54	121.54	103
19	45	94	1.00	47.00	1001.50	76.11	121.11	103
20	35	80	1.00	40.00	1041.50	79.15	114.15	97
21	21	56	1.00	28.00	1069.50	81.28	102.28	87
22	17	38	1.00	19.00	1088.50	82.73	99.73	85
23	15	32	1.00	16.00	1104.50	83.94	98.94	84
24	12	27	1.00	13.50	**1118.00**	84.97	96.97	82
				1118.00				

The elimination rate constant (Kel) can be obtained from the least-square fitted terminal log-linear portion of the plasma concentration – time profile. Value of Elimination rate constant is available in various literatures and ranging from 0.070 to 0.077[17 – 20]. For this calculation value of Kel used is 0.076. For In-vivo dissolution, asymptotic values of AUC x Ke are calculated from 11 h to 20 h. The average value found to be 118.

Result & Discussion

Area under curve of modified release tablet of 25 mg single dose is calculated as1037. This is almost similar to AUC of 2 immediate release tablets which is 1128. C_{max} of Modified release tablet (66 ng/ml) lies between 80-90% of immediate release tablet (77 ng/ml). Elimination rate of both formulation is also same. Target plasma drug concentration profile on two immediate release tablet of 12.5 mg and one modified release tablet of 25 mg is shown in Figure 1

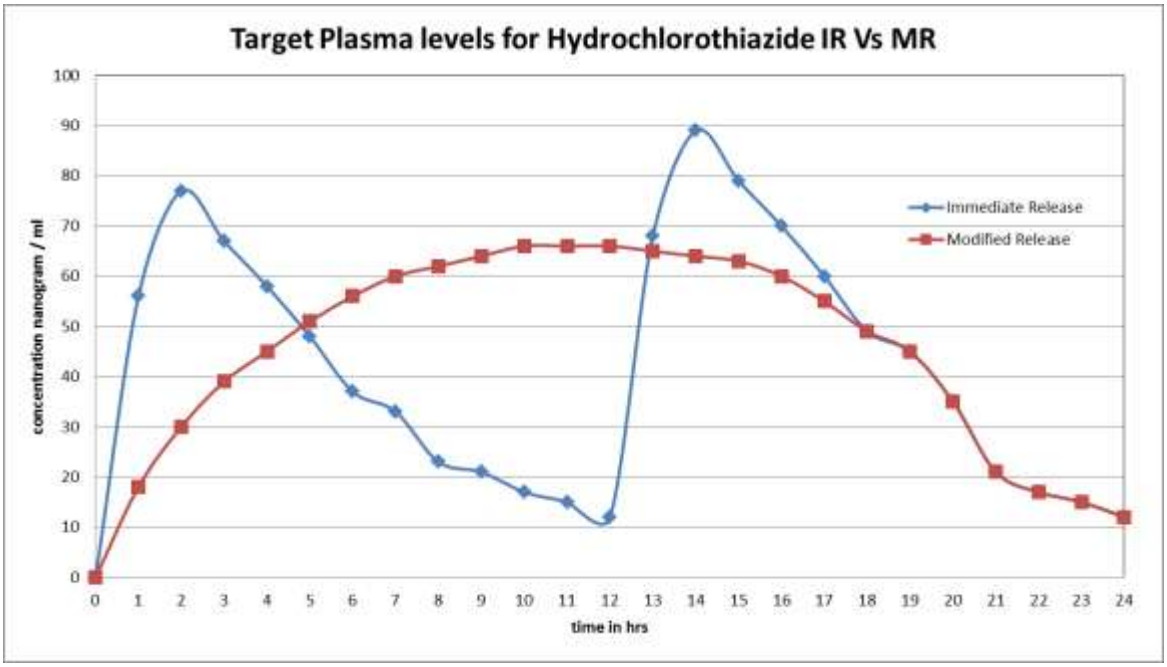

Figure 1: *In vivo* Plasma Drug concentration of Immediate Release Vs Modified Release for Hydrochlorothiazide

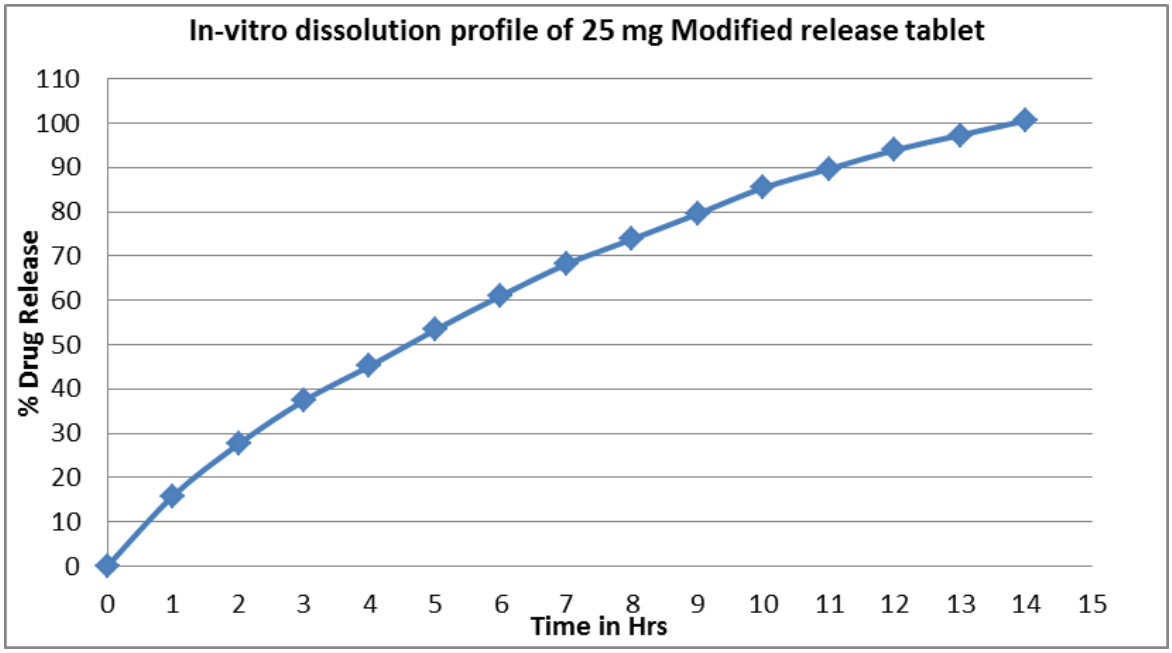

Figure 2: Required *In vitro* Dissolution profile for Hydrochlorothiazide 25 mg Modified Release Tablet

To match level A of IVIVC, point to point correlation is required. Perfect correlation is only achieved when value of slope of 1, intercept is 0 and R^2 value is 0.99 by comparing in-vivo and in-vitro dissolution. Thus required in-vitro dissolution profile is same as of calculated in-vivo dissolution. Target In-vitro dissolution to be achieved for formulation development is 0, 16, 28, 37, 45, 53, 61, 68, 74, 80, 85, 90, 94, 97 and 100 % of drug release at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 and 14 h respectively. In-vitro drug release Vs time plot is shown in **Figure 2.**

Conclusion:

Required in-vitro dissolution profile was predicted for hydrochlorothiazide modified release tablet 25 mg using Wagner-Nelson equation and deconvolution based approach. Criteria to match bio equivalence for two formulation is fulfilled i.e. AUC and C_{max}. Next step is the formulation and development of modified release tablet of hydrochlorothiazide. The similar and consistent PK/PD outcomes can be anticipated in human volunteers when tablets manufactured complying calculated dissolution profile is prepared and in-vivo study performed. This modified release tablet of Hydrochlorothiazide can be given alone or in combination with any of the beta blockers or other antihypertensive drugs as prescribed by physician. The synergistic effect will enhance pharmacological action of both drugs.

Abbreviations

JNC: joint national committee; BCS: biopharmaceutical classification system of drug; FRD: fraction of drug dissolved; FRA: fraction of drug absorbed; IVIVC: in-vitro in-vivo correlation; AUC: area under curve; Cmax: maximum concentration of drug; Tmax: time to reach maximum concentration; IR: immediate release; HCTZ: hydrochlorothiazide; $T_{1/2}$: half-life of drug; Conc: concentration; Kel: elimination rate constant; PK: pharmacokinetic; PD: pharmacodynamics; USFDA: united states food and drug administration; API: active pharmaceutical ingredient.

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